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TISSUE CULTURE AND MICROPROPAGATION FOR FOREST BIOMASS PRODUCTION: RECOMMENDATIONS FOR A CANADIAN PROGRAM

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TISSUE CULTURE AND MICROPROPAGATION

FOR FOREST BIOMASS PRODUCTION:

RECOMMENDATIONS FOR A CANADIAN PROGRAM

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November 1984

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Frank Maine Consulting Ltd.

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TISSUE CULTURE AND MICROPROPAGATION FOR FOREST BIOMASS PRODUCTION: THE CANADIAN PROGRAM AND RECOMMENDATIONS

J.A. Carlisle, E. Mason, and F.W. Maine

1. EXECUTIVE SUMMARY

A detailed literature review of tissue culture for the purpose of micropropagation of forestry species, concentrating on the last ten years in which advances have been most dramatic, was done. Visits were made to Canadian laboratories involved in any aspect of tissue culture work in forestry. Visits were also made in the United States and information was gathered on work done elsewhere in the world. From this information base, discussions were held with the principle researchers in this field in Canada, and some degree of consensus was reached on the establishment of a national R and D program and the expansion of the current effort in forest tree tissue culture.

The justification for this program is based on the following points: there is general consensus that there are impending shortages of forestry biomass; there will be increased competition internationally from conventional forestry; there will be increased use of the forestry resource for fuel and chemicals; and there is the need for increased productivity. Clonal propagation is one of the most promising methods to address these problems since this method enables the regeneration of Canadian forests with elite trees. Also, other tissue culture techniques can be extremely powerful tools for tree breeders to achieve tree improvement goals.

There are several areas where key technical problems must be resolved to realize the great potential gain from using the tissue culture techniques which are a stepping stone into more complex and powerful biotechnology tools. Fundamental research is needed to establish methods of regenerating superior plantlets from cell cultures and for applying recombinant DNA technology. These more basic problems are most suitably tackled in universities and at the National Research Council of Canada (NRC) laboratories. Certain industrial partners capable of doing applied research will provide the necessary links between laboratory research and its practical application.

In relation to the use of tissue culture techniques for micropropagation which could be tested in forestry now, the high priority area to be addressed is the process of commercialization of the technology. Crucial links in the implementation chain must be identified and problems solved. A major task that must be addressed is the economic justification of using plantlets derived from tissue culture as opposed to current practice.

Different species have different problems; therefore, it is recommended that case studies of seven species be undertaken, using available information, and the best two or three be selected for further intensive practical research that will form the basis of a national program. This national program would be designed to use the scientific resources available in Canada to solve the problems involved in generating improved trees using tissue culture methods, and by greenhouse trials and field trials assist in the transfer of this technology into economically viable commercial practice.

2. BACKGROUND INFORMATION ON THE APPLICATION OF TISSUE CULTURE METHODS TO FORESTRY

2.1 Plant Tissue Culture

Plant tissue culture refers to the growth and development of segments, tissues or cells isolated from a plant and grown under chemically and physically defined conditions in the laboratory. In addition to being a research tool in the study of cell physiology and biochemistry, tissue culture is being used to regenerate whole plants, including several species of forest trees. The results of recent laboratory work and field testing of plantlets (planting stock raised by tissue culture) have demonstrated that the technique may provide a viable alternative to the use of seedlings or cuttings for mass propagation of superior forest trees.

Micropropagation is the term used to refer to the propagation of plants raised by tissue culture methods. Not all methods are sufficiently advanced for practical application to the micropropagation of forest trees. The term presently applies in forestry to the propagation of plantlets which originate from multiple shoots induced to grow directly from plant segments such as cotyledons, buds or primary needles, and which are subsequently rooted. This method is currently being used in large-scale projects in New Zealand and the United States.

While regeneration into plantlets by other tissue culture methods using single cells or callus (mass of cultured undifferentiated cells) is theoretically possible, this has so far been achieved with very few forest tree species. The regeneration of trees by these types of tissue culture methods will have great potential application when it becomes possible to detect desirable characteristics in the cultured cells, such as resistance to disease toxins or to environmental conditions such as extreme cold. As these methods are developed, field trials will be necessary to show that these characteristics are also expressed in the regenerated mature tree. In addition, regeneration from protoplasts (cells without cell walls) will be necessary to utilize recombinant DNA technology for the transfer of genes conferring disease resistance, herbicide tolerance, nitrogen-fixation ability or other desirable traits, or to obtain somatic hybrids by fusing cells together. It has been suggested that the most convenient way to supply the vast number of plantlets needed in forestry would be by embryogenesis in cell suspension cultures. The embryogenesis process *in vitro* mimics the development of an embryo in a seed and therefore eliminates the need for multiple steps associated with the organogenesis process described above for micropropagation (i.e., the induction of buds, shoots and roots in separate phases).

2.2 Propagation of Additive and Non-Additive Genetic Traits

Micropropagation is a method of vegetative propagation and therefore produces clones of the parent plant in which both additive and non-additive genetic effects are reproduced. Additive gene effects result from the increase in the number of genes controlling a specific trait; non-additive effects result from the interaction between specific combinations of genes (McKeand and Weir 1984). In an open pollinated seed orchard only the additive genetic effects can be guaranteed in the seed (half-sib seed). Since both growth traits and pest resistance have many non-additive variance components associated with them, the total genetic potential of these important characteristics may not be captured in the seed. Controlled pollination, the artificial transfer of pollen from one flower to another so that both parents are selected (full-sib seed) to guarantee greater improvement, is difficult to carry out on a sufficiently large scale to produce the quantities of seed required in reforestation. The total genetic gain possible in a plant is therefore more easily and quickly propagated on a large scale by vegetative means rather than by seed.

2.3 Micropropagation from Seedling and Mature Explants

Commercial quantities of seedlings from a seed orchard are not available for 10-15 years after the selection of superior parent trees. According to Zobel and Talbert (1984), the lack of suitable seed is one of the greatest deterrents to reforestation. By using tissue culture methods, a large number of superior clonal plantlets can be produced from a single genetically improved half-sib or full-sib seed from a seed orchard. In New Zealand, where a program for the micropropagation of radiata pine from cotyledons of embryos from the seed of superior families has been underway since 1979, an average of 257 plantlets per seed have been produced in six months for nursery planting (Smith 1984). At present, regeneration in many species is most successful from embryonic or seedling explants and, based on the results of laboratory scale work, could now be reliably used to increase the yields from the scarce supply of improved seed of several forest trees in Canada.

In the United States techniques for propagation from explants of adolescent trees which have demonstrated their genetic potential are close to commercial application for some species such as Douglas fir. This would permit the clonal propagation of a superior tree identified in a breeding orchard.

2.4 Vegetative Propagation by Cuttings

At the same time that micropropagation techniques have been developing, those for vegetative propagation by cuttings have also been improving. Propagation by cuttings is also easier from juvenile rather than mature

plants, and clonal plantations of black spruce are being established in Northern Ontario (Phillion 1982, Libby and Rauter 1984), with 140 ramets (propagules/clone) originating from seedlings of full-sib (both parents are known) origin. However, most conifers are difficult to propagate by cuttings and the potential number of plantlets obtainable by tissue culture methods is much greater than the number of cuttings usually available from a suitable donor. Moreover, the use of cuttings is limited to clonal propagation whereas tissue culture methods can also be used for tree improvement by the exploitaion of somaclonal variation or genetic manipulation by either mutation induction or by directly engineering the genes for the desired traits.

2.5 Preservation of Genetic Variation

The dangers of narrowing the genetic base and increasing the possibility of devastation by disease or adverse environment are frequently mentioned in relation to the clonal propagation of all plants. This could have more serious consequences in forestry than in agriculture because of the long time taken to develop a tree crop. However, the forest can be managed to minimize these risks and to take advantage of the benefits which result from the uniformity of the trees. The genetic base can also become restricted if large plantations are established with seeds from the same mother tree from a seed orchard with only a small number of clones. It is widely recognized that forest clonal plantations should contain a minimum number of clones to maintain genetic diversity.

It has been possible with some plants to keep tissue explants and cell cultures at ultralow temperatures and then to regenerate plants from them (Tisdall 1984). The development of this technique (cryopreservation) for forest trees will provide a method for the preservation of valuable germplasm in addition to seed, and a means of storing material for vegetative propagation until it is needed.

2.6 The Application of Tissue Culture in Canadian Forestry

In 1984, between 300 and 400 million seedlings were planted in Canada. Less than 1% are genetically improved. In Brazil, genetic improvement of seed over several decades doubled yields of Eucalyptus and resulted in better wood and tree form. More recently, by the additional use of vegetative propagation, further genetic improvements have been made and propagation capacity increased (Zobel 1980, Durand-Cresswell, Boulay, and Franclet 1982). Similar methods, when applied to Canadian species, have the same potential. Vegetative propagation by tissue culture will be a valuable tool in achieving these improvements in a short time and in supplying the the millions of trees required for reforestation.

Tissue culture research relating to forest trees is being carried out in several locations in Canada; these were visited to obtain the information required for this survey. The practical application of micropropagation in forestry, in the light of information on large-scale projects in other countries, is assessed in this report, recognizing that potential gains are also dependent on silviculture and forest management practices. The economic feasibility of micropropagation is discussed, and problems, knowledge gaps and research needs are identified. A general strategy and recommendations for the support of an integrated Canadian program are presented.

3. PROJECTS IN MICROPROPAGATION IN CANADA AND OTHER COUNTRIES

Tissue culture techniques are being used in many countries for the improvement and propagation of agricultural and horticultural species in commercial ventures. Their application in forestry is more recent and progress has been slower, since a much longer period of time is required to assess the results of research with trees as compared with species which are harvested annually. In addition, since supplies of wood for timber and the pulp and paper industries in Canada have been adequate, there has been no urgency to increase yields by propagating genetically improved species on a massive scale. Now there is consensus both in industry and in Canada generally of impending shortages of suitable wood at a time when the renewable forest resource is becoming more important for expanded use for fuel and chemicals along with its traditional use as fibre. The integration of the production of fibre, fuels and chemicals could give the added incentive for the revitalization of Canadian forests and to increase their yield.

Fruit trees are propagated using tissue culture methods in many countries in Europe and North America, including Canada. These trees are angiosperms (deciduous trees), which are easier to regenerate by tissue culture than the gymnosperms (cone-bearing trees). Among forest trees, the angiosperm poplar has been multiplied on a commercial level in Germany, the United States, Korea and New Zealand and eucalyptus in Australia and France (Aitken-Christie 1982).

However, the forest industry is based mainly on the use of conifers which cover vast areas of land unsuited to most deciduous trees, and therefore great efforts are being made to regenerate several conifer species by commercially viable tissue culture methods. Thirty-five thousand plantlets of *Sequoia sempervirens* (redwood), which is recognized as an easy conifer to culture, had been produced in the micropropagation laboratories of Afocel (Association Forêt-Cellulose) in France by 1982. The largest scale programs on micropropagation of other conifers have been established by the Forest Research Institute in Rotorua, New Zealand (Smith 1984), and in the United States by the Weyerhaeuser Company in Tacoma, Washington. These programs are summarized below.

3.1 Micropropagation in New Zealand

The timber industry in New Zealand is based on *Pinus radiata* D. Don, which is harvested between 25 and 30 years of age. In 1951 a program was begun to obtain improved seed so that the initial planting density of seedlings necessary to ensure a final stand of 200-300 trees/hectare of high quality trees from seed of wide genetic origin could be reduced. From 1500 to 2000 seedlings were necessary to ensure this final number of suitable trees after 10 years growth. The results of the breeding program showed that

controlled pollination was necessary to capture the full genetically determined growth potential of the species (Smith 1984). The reduction in planting stock required by using genetically superior radiata pine has saved NZ $12 000 on 20 000 hectares, NZ $400 000 on pruning, and increased pulpwood yield has saved NZ $100 000 (Powledge 1984). Since it is expensive and time-consuming to produce large quantities of control-pollinated seed, in 1979 a program was established to vegetatively multiply the seed using the tissue culture method as developed by Reilly and Washer, who had first succeeded in regenerating plantlets from radiata pine embryos in 1977.

The method involves the initiation of buds and shoots on the cotyledons of mature embryos which are separated and multiplied *in vitro* and then can be immediately placed under root initiation conditions or kept in cold storage until needed. Shoot initiation and preparation for rooting require a very precise series of operations, involving several transfer steps, in specific culture media under controlled temperature, defined photoperiod and sterile conditions. Root induction similarly requires controlled conditions after which rooting continues in a non-sterile rooting medium in a moist, temperature-controlled atmosphere. The resulting plantlets are gradually acclimatized to tolerate the lower humidity and sunlight to which they will be exposed in the nursery. In early trials about 150 plantlets/seed were obtained for nursery planting; this represents the survival of about 60% of the shoots obtained after 6 months in culture. In a large scale operation this value is expected to reach 90% (Smith 1984). It is therefore possible to multiply the genetic gain in a very short time from the available seed rather than wait about 10-15 years for that seed to mature into a seed-bearing tree.

3.2 Micropropagation in the United States

Tissue culture methods for the regeneration of two economically important species in the United States, loblolly pine and Douglas fir, have been studied by several groups, and large-scale programs in micropropagation have been established. In 1983, 4000 loblolly pine plantlets were already being field tested at 13 locations in the southeastern United States as a result of work carried out at North Carolina State University in cooperation with personnel from forestry centres and industry.

Since 1974, the Weyerhaeuser Company in Tacoma, Washington, has supported research on the regeneration by tissue culture of genetically improved Douglas fir and loblolly pine, which was originally most successful using embryonic or seedling explants. Recently, a program for the large-scale micropropagation of these species from explants of older trees grown from full-sib seed was established. Plus trees are selected at 9 years of age

for Douglas fir and 6 years of age for loblolly pine, and rejuvenation is induced by spraying dormant shoot tips with the hormone benzyl adenine in the spring. Explants of these treated shoot tips are established in culture and treated to produce multiple buds and shoots which can then be individually rooted by methods similar to those used in the micropropagation of radiata pine. Culturing of explants from conifers of this age, without hormone pretreatment or other measures to induce the formation of juvenile tissue, is not usually possible. About 10 000 Douglas fir plantlets from tissue culture, and 5000 loblolly pine plantlets, which at present appear to be physiologically and morphologically normal, have been planted in the last 4 years. An economic analysis of the production process is presently being carried out.

3.3 Micropropagation in Canada

Kelowna Nurseries in British Columbia is the only commercial establishment in Canada at present using large-scale micropropagation for woody species. Techniques were originally developed for the propagation of fruit trees and ornamentals, and are now being developed for the micropropagation of conifers. Regeneration from mature Douglas fir and white spruce is being investigated by Dunstan and Mohammed of Kelowna Nurseries in cooperation with Thorpe at the University of Calgary.

The survey by Frank Maine Consulting Ltd. (FMCL) shows that there are other groups across Canada involved or interested in tissue culture research who would be capable of implementing programs in micropropagation of conifers and are eager to do so. The major centres where the work could be carried out are discussed in Chapter 5.

3.4 Economics

At present, there are no detailed studies on the economics of tissue culture produced plantlets. There is a need to have data that compare conventional methods with micropropagation, especially if the industry is to be convinced to change their current practice. The best information available today is the economic data prepared on the New Zealand work with radiata pine. It shows that, whereas the cost of producing plantlets using tissue culture is ten times that of producing plants from seed, the net return when using tissue culture produced stock is higher (Smith 1984). It should be noted that the cost of producing a seedling of radiata pine in New Zealand is much cheaper than producing seedlings of spruce or Douglas fir in Canada, since most of their production from seed is outdoors in nursery beds in contrast to the use of greenhouses in Canada. Weyerhaeuser information suggests that for Douglas fir tissue cultured plantlets cost about two to three times more than conventional seedlings. It has been suggested by Weyerhaeuser that clonal propagation of genetically superior

stock through tissue culture is economic on the better forest sites and when the trees are designated to higher value uses. However, this does not take into consideration some of the longer term benefits of clonal propagation of genetic traits such as disease resistance, herbicide tolerance, cold tolerance and a number of other desirable characteristics. Also it is important to note that the cost of production of either seedlings or tissue cultured plantlets is supposed to be minor compared to the total regeneration cost.

There is general yet strong evidence to indicate that there are significant gains to be made using tissue culture for micropropagation. Devitt (1983) of Pacific Forest Products indicated some of the ways to measure this economic gain and how to apply it to produce an economic justification for changing current practice. This technique, or one similar to it, should be used to produce the economic information necessary to justify the commercial viability of using tissue cultured plantlets to those who will implement this technology.

4. PROBLEMS, KNOWLEDGE GAPS AND RESEARCH NEEDS IN FOREST TREE TISSUE CULTURE

4.1 Micropropagation Using Current Techniques

Although conditions for regeneration by tissue culture are species-specific, the general approach developed for the micropropagation of radiata pine, Douglas fir and loblolly pine in New Zealand and the United States, discussed in sections 3.1 and 3.2, could be applied to several conifer species in Canada now, based on results obtained in research establishments here. Plantlet production programs are feasible, using explants of genetically improved seedlings and explants of rejuvenated tissue from mature trees.

To improve on the American and New Zealand methods, and ensure the success of a Canadian program, work is needed in the following areas:

1. efficiency of procedures, e.g.,

   - establish automated media preparation and automate other labour intensive steps

   - improve methods of maintaining sterile conditions

   - improve operator-dependent steps such as shoot separation

   - improve plantlet handling procedures for acclimatization and nursery planting

2. increase number of shoots rooted successfully with good root structure

3. continue to investigate ways to regenerate mature trees including fundamental understanding of the physiology and biochemistry of regeneration and its genetic regulation

4. develop cryobiology for long term storage of vegetative material

5. carry out economic analysis of micropropagation from information gained from field trials of micropropagated superior trees

6. establish procedures to implement the technology in forestry, i.e., distribution of the numbers of families of clones, adequate silvicultural practices, etc.

7. strengthen existing tree breeding programs for production of superior full-sib trees and continue to develop a "library" of superior trees from the natural forest

4.2 Future Applications of Tissue Culture

The technology of tissue culture is more than just a method for rapid propagation of scarce and valuable material. It offers great potential for speeding up tree breeding programs for the production of improved varieties. In order to select cells in culture which will grow into trees with specific characteristics, to obtain somatic hybrids not possible through conventional breeding, to apply recombinant DNA technology for the introduction of desirable genes, and then to mass propagate these superior genotypes, research will be needed in the following areas:

1. regeneration of trees from callus and cell suspension cultures, of diploid or haploid origin, by organogenesis or embryogenesis

2. control of somaclonal variation in culture either for clonal propagation or to obtain variants

3. techniques to select for desirable traits such as resistance to disease and environmental stress, in cultured cells - selection and regeneration of these cells

4. protoplast isolation, fusion and regeneration of plantlets

5. genetic expression, chromosome mapping

6. development of vectors for gene transfer into protoplasts for introduction of desirable traits

7. development of early indicators of performance such as increased photosynthetic ability

8. increased fundamental research on all aspects of tree physiology, biochemistry and genetics

5. CANADIAN CAPABILITY IN FOREST TREE TISSUE CULTURE

According to the results of the survey by Frank Maine Consulting Ltd. (FMCL), work in tissue culture and in related fields is underway at seven locations in Canada. These include:

British Columbia	- Kelowna Nurseries Ltd.
Alberta	- University of Calgary
Saskatchewan	- Plant Biotechnology Institute
Ontario	- Petawawa National Forestry Institute
	- Ontario Ministry of Natural Resources
Quebec	- University of Laval
Maritimes	- Maritimes Forest Research Centre

These programs, which are summarized in this section, should be expanded to the stage where tissue culture techniques could be applied to improve Canadian forests.

5.1 University of Calgary, Calgary, Alberta

The main centre involved in the micropropagation of forest trees, which has techniques sufficiently developed for testing in forestry, is the Department of Biology at the University of Calgary. The leading scientist in the group, Thorpe, has cooperated with New Zealand scientists on the regeneration of radiata pine which, as mentioned in section 2.1, is being propagated on a large scale in that country. Reproducible methods for plantlet formation of the Canadian species, white and black spruce, lodgepole pine and Western red cedar, have been established. Protoplast isolation of coulter pine, a technique which will be necessary for future use in tree improvement programs, has been achieved. In addition, fundamental work on the biochemical and physiological processes involved in tissue culture, which could result in improvements over the present empirical methods, is being carried out.

5.2 Kelowna Nurseries, Kelowna, British Columbia

The group at the University of Calgary is cooperating with Dunstan and Mohammed of Kelowna Nurseries in British Columbia on the micropropagation of mature Douglas fir and white spruce by rooting of multiple shoots produced from bud and shoot apex explants. This research could lead to

commercial production of these species. The nursery is currently producing commercial quantities of superior fruit trees and ornamentals using tissue culture.

5.3 Plant Biotechnology Institute (PBI), Saskatoon, Saskatchewan

The Plant Biotechnology Institute (PBI) of the National Research Council is involved in tissue culture research relating to agricultural, horticultural and pharmacological applications, and recently tissue culture of forest trees has been added to the program. Work is underway to develop methods of achieving suspension cultures and regeneration of whole plants from single cells of forest tree cultures, drawing on the wide experience in methods of plant tissue culture of the group headed by Constabel.

The work of Kartha in cryobiology and in developing *in vitro* methods for selecting cold tolerant plants is expected to have important applications for tree improvement programs.

5.4 Petawawa National Forestry Institute (PNFI), Chalk River, Ontario

The Petawawa National Forestry Institute is currently in the process of expanding its forest biotechnology program to include tissue culture. For many years the PNFI, which is the location of the National Seed Bank, has been involved in tree genetics and breeding programs, while their plant biotechnology program has been focused on nitrogen fixation in forest trees. Based on the experience of its personnel and because of its facilities, which include laboratories, greenhouses, nurseries and land for field planting, PNFI has identified the following areas for its involvement in tissue culture:

- practical evaluation and application of tissue culture methods
- early performance and trials of micropropagated plantlets
- testing of improved clones
- refinement of greenhouse and field transfer methods
- supply/improvement of stock of control pollinated seed for proliferation by tissue culture
- development of molecular genetics techniques and expansion of genetic studies to include chromosome mapping and information on genetic expression
- development of early diagnostic methods for determining performance, e.g., enzyme or genetic markers, morphological characteristics

- development of methods of screening for disease and herbicide resistance at the cellular level

5.5 Ontario Ministry of Natural Resources (OMNR), Maple, Ontario

Ho, at the OMNR facility in Maple, has regenerated poplar from seedling explants and from haploid anther cultures using tissue culture techniques. There is a possibility that programs presently being carried out at Maple may be dispersed to other centres in Ontario in the future, but it is important that the work on haploid culture be continued to provide genetic information and homozygous trees for breeding purposes.

5.6 University of Laval, Forest Biology Research Centre, Ste-Foy, Quebec

Work is in progress on the micropropagation of alder species at the University of Laval. The main scientists in the group working in these areas are Lalonde and Fortin. Research is also being carried out on the tissue culture of birch and larch.

A long term goal is to transfer the symbiotic nitrogen-fixing ability of alder to birch using protoplast fusion; this will involve genetic as well as regeneration studies.

5.7 Maritimes Forest Research Centre (MFRC), Fredericton, New Brunswick

Tissue culture research began at MFRC in 1970 in cooperation with the tree improvement program begun in 1954. Work has been carried out by Bonga on the tissue culture of explants from mature trees and on haploid cultures. At present, the possiblity of using tissue culture methods for the multiplication of larch hybrids created by the tree breeders, which produce few seeds and can provide a limited number of cuttings, is being investigated. Work is also planned using genetically improved spruce and pine.

6. STRATEGY FOR THE IMPLEMENTATION OF TISSUE CULTURE IN FORESTRY IN CANADA

From the survey conducted by FMCL, it is apparent that a national program could be developed in applied tissue culture based on the current strengths of scientists already working in this area. With the cooperation of forestry personnel, this work would make a valuable contribution to the forest industry in Canada.

The major features of a strategy to implement such a program are the following:

1. Seven establishments in Canada, mentioned in Chapter 5, already have the capacity to be involved in a forestry tissue culture program. These are located across the country from British Columbia to the Maritimes. To make the best use of this current strength, a network could be established with two focal points, PNFI and PBI. PNFI would be the major focus for applied work and PBI the focus for fundamental research. Since there are regional differences in the requirements of the forest industry, the work at smaller establishments across the country rather than at a single centre could reflect these different needs, as well as being a practical way of building on existing experience and facilities.

   Other groups or individuals involved in tissue culture relating to forestry, apart from those already mentioned, should be added to the network as soon as possible, e.g., Zsuffa and Hubbes at the University of Toronto, and Webb at Queen's University, Kingston.

2. The work most important to Canada must be defined, and the scientific and economic barriers to practical application identified and tackled. Methods of commercializing tissue culture technology must be established. This includes technology transfer such as the transfer of present tissue culture techniques from the lab to greenhouse and field evaluation leading to a demonstration program of substantial clonal forest plots of micropropagated stock.

3. A gradual increase of applied and fundamental research, to improve present methods and to develop the technology to its full potential, will enable Canada to compete in both economic terms and time scale of implementation.

4. With the existing experience in tissue culture in Canada, it should be realized that the opportunity exists not only to establish a national strategy to utilize this technique in forestry, but also to become a world leader in the field.

7. RECOMMENDATIONS

From the study by FMCL, it has been concluded that existing tissue culture work in Canada could become the basis of a national program for the application of tissue culture in forestry. To initiate the program, the following recommendations are made:

1. - PNFI will become the focus of applied tree tissue culture technology for groups across the country.

   - PBI will similarly become the focus of fundamental research.

   - This program will require expanded financial support and would be implemented through in-house work at PNFI and PBI; through contract-supported work at industry and universities; through industrial programs such as IRAP and PILP; and through NSERC strategic grants to universities.

2. - Since micropropagation by methods similar to those being used in New Zealand and the United States could be applied to several trees in Canada now, it is recommended that case studies be carried out with selected species, as soon as possible, to identify any special problems associated with the tissue culture propagation methods of that particular species (such as the availability of genetically improved seed).

   After discussions with several of the scientists from CFS, PBI, the universities and Kelowna Nurseries, a consensus was reached that the following species should be studied to identify research needs with the view to choosing two or three for greenhouse and field trials:

Pulp and Paper Species	BLACK SPRUCE
	WHITE SPRUCE
	LARCH
Timber Species	DOUGLAS FIR
	WHITE PINE
	LARCH
Energy Species	POPLAR
	ALDER
	LARCH

   - An economic analysis would be part of each case study.

3. - For future applications of tissue culture mentioned in section 4.2, it is recommended that tissue culture scientists and tree geneticists from across the country meet to decide on a program which utilizes their talents cooperatively and is directed towards the practical needs of forestry.

4. - A mechanism for the commercialization of tissue culture in forestry must be established. It would therefore be necessary to arrange meetings between representatives from provincial governments, commercial nurseries and the forest industries to identify the barriers and to establish procedures to overcome these barriers.

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